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Pharmacokinetic Profile of Progabide, a New γ-Aminobutyric Acid-Mimetic Drug, in Rhesus Monkey

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Abstract □ The pharmacokinetic profile of progabide was investigated in five chronically catheterized male rhesus monkeys. The experimental design included single-dose intravenous bolus and oral administration at two dose levels (50 and 100 mg) and zero-order intravenous infusion for 7 days. Plasma samples were analyzed by electron-capture–GLC. Protein binding of the drug was determined by equilibrium dialysis (4°). A one-compartment open model with monoexponential decay was proposed to describe the pharmacokinetics. The mean parameters (±*SEM*) of the 50- and 100-mg iv bolus were: total body clearance, 2.09 (±0.15) and 1.53 (±0.18) liter/hr/kg; half-life, 0.656 (±0.054) and 0.789 (±0.079) hr; distribution volume, 1.97 (±0.08) and 1.79 (±0.21) liter/kg. Progabide was highly bound to plasma proteins and also to erythrocytes. The drug was rapidly absorbed (T_{max} < 1 hr at both doses). The mean bioavailability was attributed principally to a first-pass effect. In the constant rate infusion study, systemic clearance was larger than that of the single dose studies.

Keyphrases □ Progabide—pharmacokinetics in rhesus monkeys, plasma bioavailability □ Bioavailability—pharmacokinetics of progabide in plasma of rhesus monkeys □ Pharmacokinetics—bioavailability of progabide in plasma of rhesus monkeys □ Electron-capture–GLC—determination of progabide in rhesus monkey blood plasma, bioavailability, pharmacokinetics

Progabide[1] is a new γ-aminobutyric acid-mimetic drug with a broad spectrum of anticonvulsant activity (1–6). Progabide was designed to be a carrier of γ-aminobutyramide into the central nervous system where it would be broken down and release γ-aminobutyramide.

The present study was undertaken to characterize the pharmacokinetic behavior of progabide in the rhesus monkey in view of its efficacy testing in the chronic epileptic monkey model.

EXPERIMENTAL

Five healthy male rhesus monkeys (*Macaca mulatta*) (2.7–3.8 kg) were used in this study. The monkeys were maintained on fresh fruits and monkey chow and chaired for the duration of the study. Each monkey had two chronic catheters, one for drug administration (femoral vein) and one for blood sampling (jugular vein). The experimental design included single-dose and chronic administration studies. In the single-dose studies, each monkey received 50 or 100 mg of the drug intravenously and orally in a randomized fashion. The drug was dissolved in 100% polyethylene glycol 400[2] at concentrations of 25 and 50 mg/ml. Two milliliters of this solution was injected over 5 min through the femoral vein or administered by intranasal gastric intubation. Fourteen (50-mg dose) or 15 (100-mg dose) blood samples were collected over a 5–6-hr period. Ethylenediaminetetraacetic acid tripotassium salt was used as an anticoagulant, and the blood samples were stored in ice. They were centrifuged at 4° and the separated plasma was frozen immediately. The samples were kept frozen until assayed. Some blood samples were also frozen to determine the blood level of the drug and the total blood clearance in each monkey.

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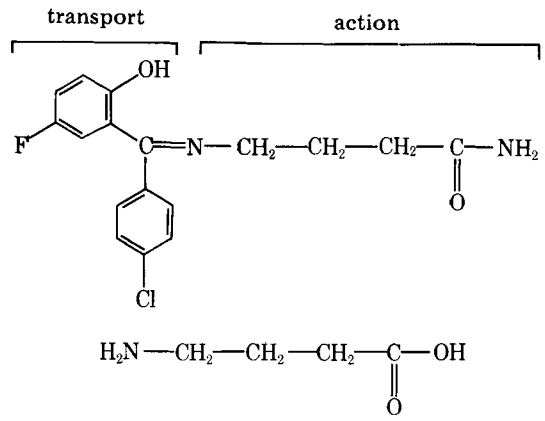

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The chronic administration studies consisted of constant rate intravenous infusion for 7 days. The drug was dissolved in 80% polyethylene glycol 400 at a concentration of about 3 mg/ml (1.91–3.84 mg/ml), and the infusion rate was 1 ml/hr. Blood samples were collected during the accession to steady state (0–6 hr), on a daily basis during steady state, and during the post steady-state decay phase.

Protein binding of progabide was measured by equilibrium dialysis at 4° to minimize the degradation of drug in plasma. A volume of 0.7 ml of pooled monkey plasma, and an equal volume of isotonic phosphate buffer system (pH 7.4), were used. The dialyzing system consisting of plexiglass cells was rotated at a rate of 10 rev/min for 12 hr. The dialysis was carried out with spiked plasma samples with concentrations ranging from 0.4 to 34.6 μg/ml (determined postdialysis). The drug on both sides of the membrane was analyzed by electron-capture–GLC.

Progabide concentrations were determined by the method of Gillet and Dring[3] using a GLC equipped with electron-capture detector[4]. The column consisted of 3% OV-17 on Chromosorb W-HP (column length was 184.3 cm and 2-mm i.d.). Temperatures of detector, injection port, and oven were 300, 250, and 240°, respectively. An analog of progabide, [α-(chloro-4′-phenyl)chloro -5- hydroxy-2-benzylideneamino]-4-butyramide, was used as the internal standard.

Areas under intravenous and oral curves were calculated by the trapezoidal method (with extrapolation to infinite time). Systemic clearance was determined by dose–area ratio. Oral bioavailability was calculated by ratio of oral and intravenous areas.

[1] Synthélabo-L.E.R.S., Paris, France.
[2] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[3] Unpublished method, 1978.
[4] Model 5710A, Hewlett-Packard, Palo Alto, CA 94304.

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Table I—Pharmacokinetic Parameters of Progabide in the Monkey following Intravenous Bolus Administration at Two Doses [a]

Monkey Number	Body Weight[b], kg	Systemic Plasma Clearance, liter/hr/kg		Volume of Distribution, liter/kg		Half-Life, hr	
		50 mg	100 mg	50 mg	100 mg	50 mg	100 mg
564A	3.15	2.30	—	1.85	—	0.552	—
514A	3.47–3.77	1.96	1.70	1.81	1.47	0.643	0.556
706A	2.65–3.14	2.11	1.92	2.27	2.33	0.729	0.838
574A	3.10–3.80	1.60	1.09	1.95	1.42	0.822	0.901
020	3.50–3.53	2.50	1.40	1.96	1.92	0.532	0.860
Mean		2.09	1.53	1.97	1.79	0.656	0.789
(*SEM*)		(0.15)	(0.18)	(0.08)	(0.21)	(0.054)	(0.079)
Paired *t* test ($n = 4$)		NS		NS		NS	

[a] 50 and 100 mg. [b] The two values represent weights at beginning and end of experimentation period.

Table II—Pharmacokinetic Parameters of Progabide in the Monkey following Oral Administration at Two Doses [a]

Monkey Number	Half-Life, hr		Bioavailability, %	
	50 mg	100 mg	50 mg	100 mg
564A	0.615	—	29.1	—
514A	0.704	0.594	40.3	31.9
706A	0.821	1.02	69.3	87.0
574A	0.621	0.852	49.0	33.7
020	0.685	0.804	46.5	21.8
Mean	0.687	0.818	46.8	43.6
(±*SEM*)	(0.038)	(0.088)	(6.6)	(14.7)
Paired *t* test ($n = 4$)	NS		NS	

[a] 50 and 100 mg.

Table III—Protein Binding of Progabide in Monkey Plasma

Concentration, μg/ml	Free Fraction[a]
0.42	0.020 ± 0.005
0.75	0.030 ± 0.009
1.49	0.027 ± 0.0005
3.47	0.035 ± 0.003
8.91	0.051 ± 0.001
34.61	0.049 ± 0.003

[a] Values are the mean ±*SD* of three determinations.

RESULTS AND DISCUSSION

Single-Dose Intravenous Studies—Representative semilog plasma concentration–time curves obtained for the two intravenous bolus doses in monkeys 574A and 706A are shown in Figs. 1 and 2. The continuous lines were obtained by nonlinear least-squares fitting of experimental data points to a monoexponential equation. A summary of the pharmacokinetic parameters obtained from intravenous bolus administration is presented in Table I.

Progabide has a large systemic clearance, large distribution volume, and short biological half-life. The total body clearance at the 50- and 100-mg doses ranged from 1.60 to 2.50, and 1.09 to 1.92 liter/hr/kg, respectively. The distribution volume at the same doses ranged from 1.81 to 2.27 and 1.42 to 2.33 liter/kg, respectively. The apparent half-life at the 50-mg dose ranged from 0.53 to 0.82 hr and, at the 100-mg dose, from 0.56 to 0.90 hr. Although progabide clearance decreased with dose in all four monkeys, statistical comparison showed no significant difference ($p = 0.088$). There was also no apparent dose dependency in distribution volume or elimination half-life.

The progabide blood–plasma ratio was calculated in four monkeys and the mean value (±*SEM*) was 0.700 ± 0.019. Hematocrit was also measured in three monkeys with a mean of 36% yielding a mean red blood cell–plasma ratio of 0.17. If the free fractions found *in vitro* are applicable *in vivo*, it would suggest that progabide binds to erythrocytes.

Urinary excretion of unchanged drug was negligible during 24 hr. The large plasma clearance and low blood–plasma ratio of progabide suggest that progabide has a medium to high extraction ratio in rhesus monkeys (assuming all metabolism is hepatic and nonrenal elimination of progabide is negligible).

Single-Dose Oral Studies—Figures 3 and 4 show the plasma concentration–time profiles and bioavailability obtained following two single

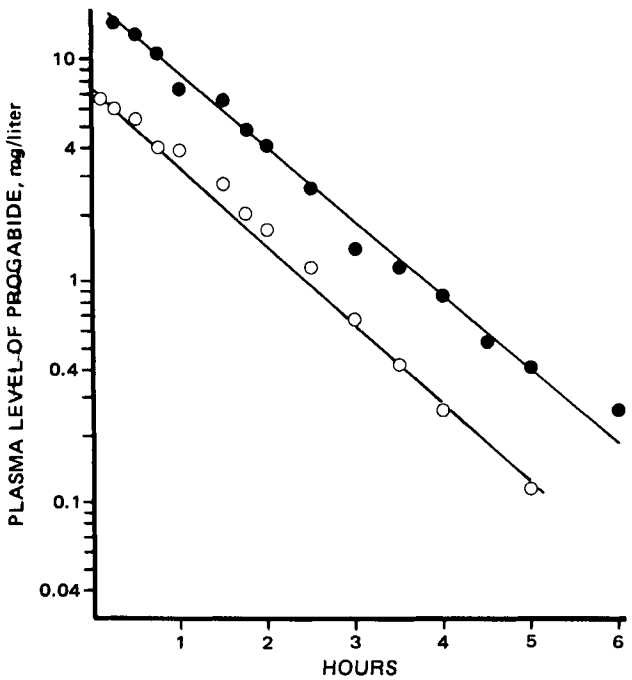

Figure 1—*Semilog plasma–time curve after intravenous bolus administration of progabide (50 mg, ○; 100 mg, ●) in monkey 574A.*

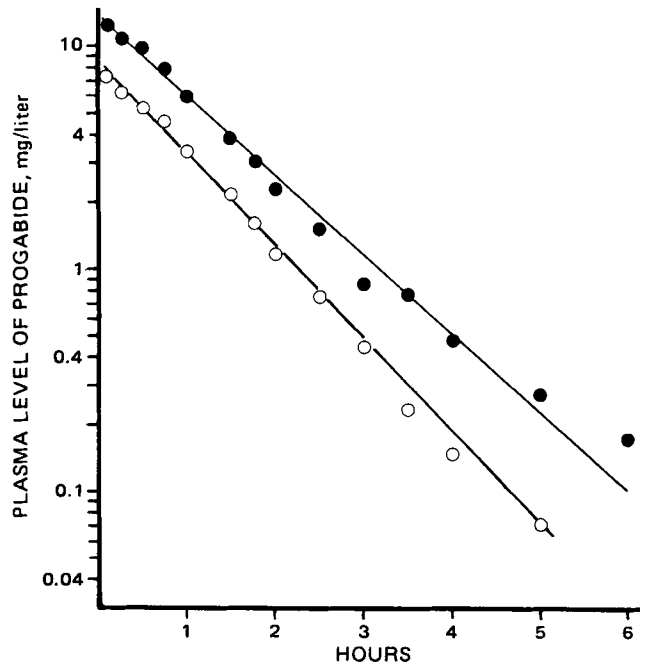

Figure 2—*Semilog plasma–time curve after intravenous bolus administration of progabide (50 mg, ○; 100 mg, ●) in monkey 706A.*

Table IV—Pharmacokinetic Parameters of Progabide Derived from Constant Rate Infusion Studies

Monkey Number	Infusion Rate, mg/hr	Steady-State Plasma Concentration, mg/liter	Systemic Plasma Clearance, liter/hr/kg	Postinfusion Half-Life, hr
514A	2.03	0.237	2.47	0.831
706A	1.92	0.314	2.31	1.028
574A	1.62	0.236	2.21	0.666
020	2.84	0.292	2.76	—
Mean	2.10	0.270	2.44[a]	0.842[b]
(±*SEM*)	(0.26)	(0.020)	(0.12)	(0.09)

[a] Using a two-way ANOVA, the levels of significance for the pairwise comparisons of infusion clearances to those of single dose studies were $p = 0.071$ for infusion *versus* 50-mg dose and $p = 0.0023$ for infusion *versus* 100-mg dose. [b] $p > 0.05$ when compared with single intravenous bolus studies using ANOVA.

oral and intravenous bolus doses in one monkey. Plasma half-life and bioavailability are tabulated in Table II. Progabide was absorbed rapidly and typically reached maximum plasma levels ~1 hr postdose. The bioavailability was between 29 and 69% with a mean (±*SEM*) of 46.8 (±6.6)% for the 50-mg dose and between 22 and 87% with a mean (±*SEM*) of 43.6 (±14.7)% for the 100-mg dose. There was no significant difference between the bioavailabilities of both doses ($p > 0.05$). The incomplete bioavailability is probably due to a first-pass effect and not to poor absorption. Based on a mean extraction ratio, the mean bioavailability was calculated to be 34%, which is in agreement with experimental findings. This calculation assumed complete metabolism in the liver with a hepatic blood flow of 13 liter/hr (7, 8).

Postabsorption half-lives ranged between 0.6 and 0.8 hr at the low dose, and between 0.6 and 1.0 hr at the high dose. These are in agreement with the corresponding intravenous data.

Protein Binding Studies—Protein binding of progabide was determined *in vitro* with plasma concentrations ranging from 0.4 to 34.6 μg/ml. Free fractions ranged between 0.020 and 0.051 (Table III) showing that progabide is highly bound to plasma proteins. Binding appeared concentration-dependent in this plasma level range.

Chronic Dosing Studies—A 7-day continuous zero-order infusion study was performed to test for the presence of any time dependency. A representative plasma concentration–time plot is shown in Fig. 5 and a summary of the pharmacokinetic parameters in four monkeys is given in Table IV. Steady state was achieved in a few hours as predicted from the single-dose studies. Steady-state plasma concentrations ranged between 0.24 and 0.31 mg/liter with infusion rates of 1.6–2.8 mg/hr. Systemic clearance values, ranging from 2.21 to 2.76 liter/hr/kg, were larger than those obtained in single-dose studies. These differences reached

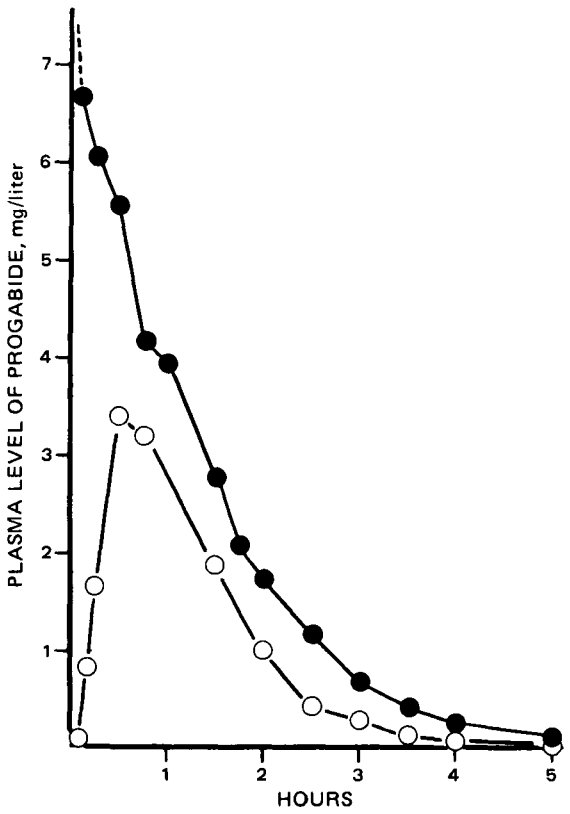

Figure 3—*Plasma concentration–time curve after the intravenous bolus (●) and oral (○) administration of 50 mg of progabide.*

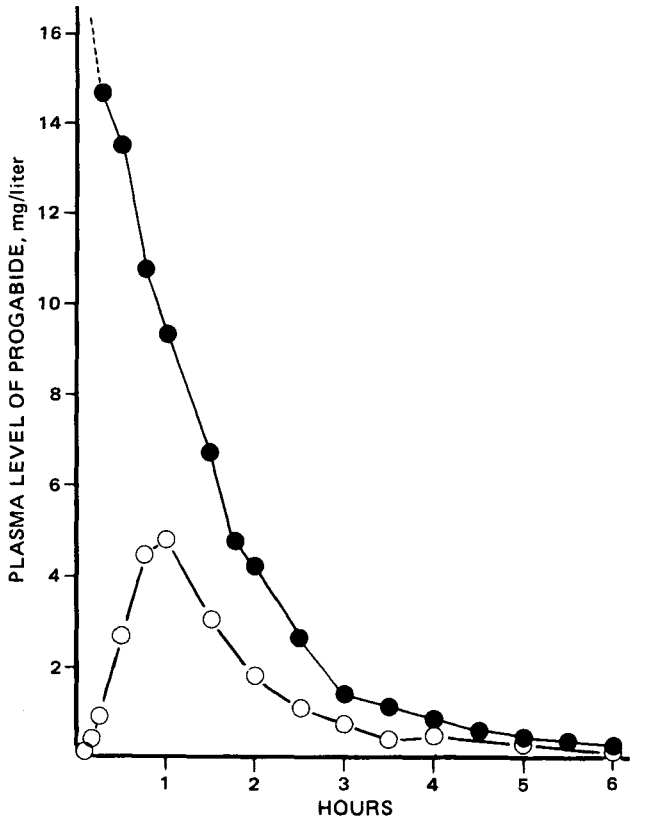

Figure 4—*Plasma concentration–time curve after the intravenous bolus (●) and oral (○) administration of 100 mg of progabide.*

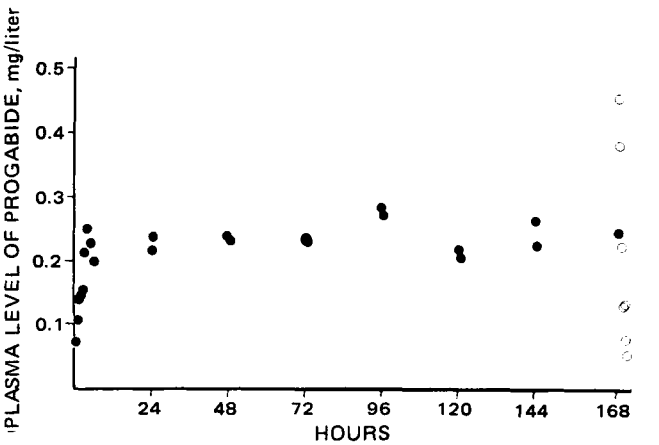

Figure 5—*Plasma concentration during (●) and after (○) the intravenous infusion of progabide in monkey 514A.*

significance for the comparison of the infusion values with the 100-mg dose. Post steady-state disappearance half-lives ranging from 0.67 to 1.03 hr were not different from the single-dose data, (the increase in the postinfusion plasma concentration seen in Fig. 5 was due to flushing of the catheter with saline at the time the infusion was stopped). No time-dependent change in clearance or half-life was exhibited over the 1-week period of the study.

In the rhesus monkey, progabide behaves as a medium extraction ratio drug with incomplete bioavailability and first-order disappearance kinetics. It exhibits nonlinearity in plasma binding *in vitro*. There was a tendency for systemic clearance to decrease within a two-fold dose range. Progabide exhibited no evidence of time dependency in clearance during chronic infusion.

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(4) G. Bartholini, B. Scatton, B. Zivkovic, and K. G. Lloyd, *Ibid.*, pp. 326.

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Determination of Ethinyl Estradiol in Solid Dosage Forms by High-Performance Liquid Chromatography

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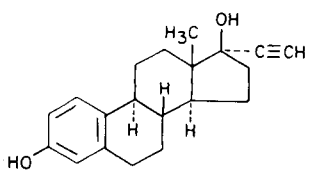
Abstract □ A rapid, reproducible high-performance liquid chromatographic system for the determination of ethinyl estradiol in solid dosage forms consisting of a reversed-phase column with a mobile phase of 0.05 *M* aqueous KH_2PO_4–methyl alcohol (2:3) and fluorescence detection has been developed. This stability-indicating method is applicable to tablets containing ethinyl estradiol alone or in combination with methyltestosterone and progesterones. The procedure has been used for the determination of ethinyl estradiol in single tablets, stability samples, and dissolution medium. Recovery of drug substance added to placebo was from 97.3 to 101.5% in stability and single-tablet assays, and 95.4 to 102.2% in dissolution assays. Reproducibility studies gave relative standard deviations of 0.4–2.2%.

Keyphrases □ Ethinyl estradiol—high-performance liquid chromatographic analysis, stability, content uniformity and dissolution assays □ High-performance liquid chromatography—analysis, ethinyl estradiol, fluorescence detection □ Estrogens—ethinyl estradiol, high-performance liquid chromatography, analysis of solid dosage forms

Ethinyl estradiol (I), a well known estrogen, is used in hormonal therapy, contraception, and certain cancer treatments. The steroid may be prescribed either alone, as in treatment of estrogen deficiency, or in combination with a progesterone in contraceptive formulations.

Current methods for the analysis of ethinyl estradiol include both wet chemical (1–4) and chromatographic methods. Among the latter are gas chromatography (GC) (5) and high-performance liquid chromatography (HPLC) with UV detection (6–10).



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Determination of ethinyl estradiol by HPLC has been hampered by low sensitivity, as the administered dosages may be as low as 10 μg/tablet. To counter this lack of sensitivity, several investigators have proposed analyzing composite samples of up to 10 tablets (7, 8). While useful for stability assays, this approach is not suitable for either content uniformity or dissolution assays. Other investigators have increased detection sensitivity by using precolumn derivatization with dansyl chloride and fluorescence detection (11, 12). A much simpler approach is to use the native fluorescence of the phenolic ring of the steroid; this method has been used for normal phase HPLC of estrogens (13) and of ethinyl estradiol in cosmetics (14, 15).

A reversed-phase HPLC system with native fluorescence detection for the determination of ethinyl estradiol in solid dosage forms is described. The proposed method, which requires minimal sample preparation, is not only stability-indicating but also sensitive enough for use in content uniformity and dissolution assays.

EXPERIMENTAL

Materials—HPLC grade methyl alcohol[1], *o*-phenylphenol[2], and potassium phosphate monobasic crystals[2] (KH_2PO_4) were obtained from commercial sources. Ultrapure water was prepared by deionization, treatment for removal of organic compounds, and filtering[3].

Apparatus—The high-performance liquid chromatograph was equipped with a constant flow pump[4], an automatic injector[5], a fluores-

[1] Mallinckrodt, Inc., Paris, KY 40361.
[2] Matheson, Coleman and Bell, Cincinnati, OH 45212.
[3] Milli-Q Water Purification System, Millipore Corp., Bedford, MA 01730.
[4] Model M6000A Chromatography Pump, Waters Associates, Milford, MA 01757.
[5] WISP Model 710A Automatic Injector, Waters Associates, Milford, MA 01757.

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